

CLAIMS

1                   1.           A method for quantitative and qualitative analysis of a nucleic  
2 acid analyte in a sample suspected to contain the nucleic acid analyte, comprising the steps  
3 of:

4                   (a)   preparing a reaction mixture containing the sample and a known amount of  
5 an internal quantitative standard

6                   (b)   combining a first aliquot of the reaction mixture with a set of amplification  
7 reagents, said reagents including a first primer pair which is effective to amplify a first  
8 region of the nucleic acid analyte if present in the sample to produce a first amplified  
9 sample fragment and to amplify at least a portion of the internal quantitation standard to  
10 produce a control fragment;

11                  (c)   amplifying the nucleic acid analyte and the internal quantitation standard in  
12 the reaction mixture using the first primer pair to produce an amplification product mixture  
13 containing first amplified sample fragments and control fragments when the nucleic acid  
14 analyte is present in the sample, and only control fragments when the nucleic acid analyte is  
15 not present in the sample;

16                  (d)   analyzing the relative amounts of first amplified sample fragments and  
17 control fragments in the amplification product mixture to quantify the amount of nucleic  
18 acid analyte in the sample; and

19                  (e)   determining the sequence of the first amplified sample fragments in the  
20 amplification mixture to determine the qualitative characteristics of any nucleic acid  
21 analyte in the sample using at least a first sequencing primer,

22                  wherein the internal quantification fragment is derived from the analyte nucleic  
23 acid by the incorporation of a plurality of sequence variations, including at least a first  
24 sequence variation effective to render the internal quantitation standard distinguishable  
25 from the first amplified sample fragment, and a second sequence variation effective to

26 substantially eliminate the production of sequencing products from interaction of the  
27 internal quantitation standard and the first sequencing primer.

1           2.    The method according to claim 1, wherein the nucleic acid analyte includes  
2 a plurality of regions of interest, and wherein the first primer pair is effective to amplify  
3 only one of these regions.

1           3.    The method according to claim 2, further comprising the step of combining  
2 one or more additional aliquots of the reaction mixture with one or more additional sets of  
3 amplification reagents, each additional set of reagents including an additional primer pair  
4 which is effective to amplify a further one of the regions of interest in the nucleic acid  
5 analyte if it is present in the sample to produce an additional amplified sample fragment,  
6 wherein the additional primers in the additional reagent sets are not effective to amplify the  
7 internal quantitation standard.

1           4.    The method according to claim 1, wherein the nucleic acid analyte is HIV-  
2 1.

1           5.    The method according to claim 4, wherein the HIV-1 nucleic acid analyte  
2 is evaluated by analysis of the protease gene, and a beginning, middle and end portion of  
3 the reverse transcriptase gene as separate regions of interest, and wherein the first primer  
4 pair is effective to amplify only one of these regions.

1           6.    The method according to claim 5, wherein the first primer pair is effective  
2 to amplify the beginning portion of the reverse transcriptase gene of HIV-1.

1           7.    The method according to claim 6, further comprising the step of combining

three additional aliquots of the reaction mixture with three additional sets of amplification reagents, each additional set of reagents including an additional primer pair which is effective to amplify one of the protease gene, or the middle or end portion of the reverse transcriptase in the HIV-1 nucleic acid analyte if it is present in the sample to produce three amplification product mixtures, each containing an additional amplified sample fragment, wherein the additional primers in the additional reagent sets are not effective to amplify the internal quantitation standard.

8. The method of claim 7, wherein the first sequence variation in the internal quantitation standard is an insertion or deletion mutation in the beginning portion of the HIV-1 gene.

9. The method of claim 6, wherein the first sequence variation in the internal quantitation standard is an insertion or deletion mutation in the beginning portion of the HIV-1 gene.

10. An internal quantification standard for use for analysis of an analyte nucleic acid. comprising a nucleic acid polymer derived from the analyte nucleic acid by the incorporation of a plurality of sequence variations, including at least a first sequence variation effective to render a control fragment produced by amplification of the internal quantitation standard with a first pair of primers distinguishable from a first amplified sample fragment produced by amplification of the analyte nucleic acid with the first pair of primers, and a second sequence variation effective to substantially eliminate the production of sequencing products from interaction of the internal quantitation standard with a sequencing primer effective for sequencing of first amplified sample fragment.

11. The internal quantitation standard of claim 10, wherein the first sequence

variation is an insertion or deletion mutation.

12. The internal quantitation standard of claim 11, wherein the analyte nucleic acid from which the internal quantitation standard is derived comprises the protease and reverse transcriptase genes of HIV-1.

13. The internal quantitation standard of claim 12, wherein the first sequence variation is an insertion or deletion mutation.

14. A kit for quantitative and qualitative analysis of a nucleic acid analyte in a sample comprising, in packaged combination:

(a) a first pair of amplification primers effective to amplify a first region of the nucleic acid analyte to produce a first amplification product;

(b) a first sequencing primer for generating sequencing fragments from the first amplification product;

(c) an internal quantitation standard, comprising a nucleic acid polymer derived from the analyte nucleic acid by the incorporation of a plurality of sequence variations, including at least a first sequence variation effective to render a control fragment produced by amplification of the internal quantitation standard with the first pair of primers distinguishable from a first amplified sample fragment produced by amplification of the analyte nucleic acid with the first pair of primers, and a second sequence variation effective to substantially eliminate the production of sequencing products from interaction of the internal quantitation standard with the first sequencing.

15. The kit of claim 14, further comprising a second pair of amplification primers effective to produce a second amplification product from a second region of the nucleic acid analyte different from the first region, and a second sequencing primer for

4 generating sequencing fragments from the second amplification product.

1 16. The kit of claim 14, wherein the nucleic acid analyte is HIV-1, and the first  
2 and second regions are selected from among regions spanning areas of sequence variability  
3 in the protease gene and regions spanning areas of sequence variability in the reverse  
4 transcriptase gene.

1 17. The kit of claim 14, wherein the first sequence variation is an insertion of  
2 deletion mutation.

1 18. The kit of claim 17, wherein the nucleic acid analyte is HIV-1, and the first  
2 and region is selected from among regions spanning areas of sequence variability in the  
3 protease gene and regions spanning areas of sequence variability in the reverse transcriptase  
4 gene.

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